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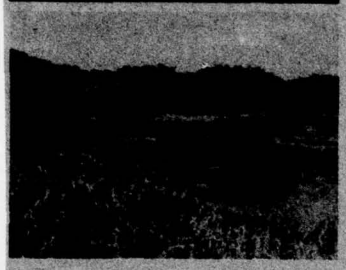

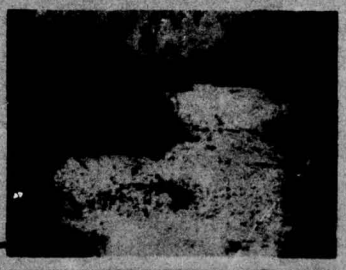

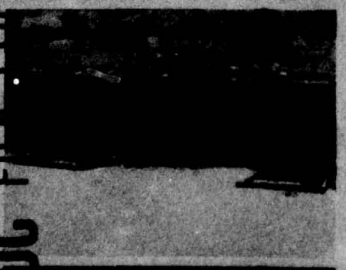

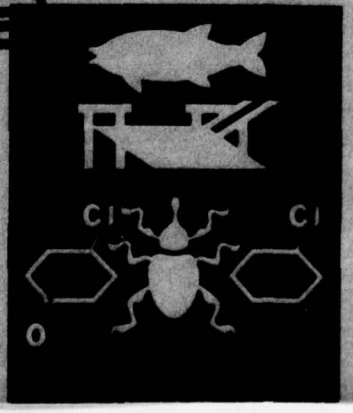

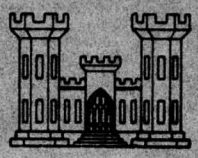

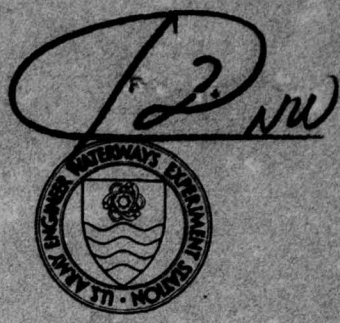

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FATE OF 2,4-D IN FISH AND BLUE CRABS

by Harish C. Sikka

Syracuse Research Corporation
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Final Report

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Very little information was available on the degree of accumulation of dimethylamine (DMA) salt of 2,4-dichlorophenoxyacetic acid (2,4-D) by fish and blue crabs. Such knowledge ~~was~~ is important to the process of registering 2,4-D in slow moving waters for aquatic plant control. ~~In order~~ To assess the ability of fish to uptake and metabolize 2,4-D, ~~this study was conducted using~~ bluegills and channel catfish under conditions where ~~the~~ microbial degradation of the herbicide in water was minimal. Also, to determine the residues of 2,4-D in

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20. ABSTRACT (Continued).

crab-meat, blue crabs were collected from four locations along the St. Johns River in Florida, following treatment of waterhyacinths with the 2,4-D DMA. The results of this study demonstrated that the herbicide did not bioaccumulate in fish and did not exceed the established tolerance limit (1.0 ppm) in blue crab flesh.

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Preface

The study reported herein was conducted under Contract No. DACW39-74-C-0068. The work was administered under the direction of the Mobility and Environmental Systems Laboratory of the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Mississippi. Dr. H. C. Sikka, Manager of the Pesticides and Toxic Substances Laboratory of the Syracuse Research Corporation, prepared the report. Mr. W. N. Rushing was the Contracting Officer's representative; his assistance and constructive criticism is hereby acknowledged.

Director of the WES during the preparation and publication of this report was COL J. L. Cannon, CE. Technical Director was Mr. F. R. Brown.

Contents

FATE OF 2,4-D IN FISH AND BLUE CRABS

Uptake and Metabolism of Dimethylamine Salt of 2,4-D by Fish

Introduction

1. The dimethylamine (DMA) salt of 2,4-dichlorophenoxyacetic acid (2,4-D) is used extensively for controlling aquatic plants, such as waterhyacinth and Eurasian watermilfoil. This formulation of the herbicide is relatively nontoxic to fish; the 96-hr TL_{50} values for bluegills (Lepomis macrochirus) and channel catfish (Ictalurus punctatus) are 160 and 125 ppm, respectively.[1] A knowledge of the degree of accumulation of this herbicide by fish is important if they are to be used for human consumption. The herbicide, if accumulated by fish, may undergo metabolic transformation. The nature of these metabolites must be known in order to assess their possible toxicity to fish and man.

2. Presently, very little information is available on the uptake and metabolism of 2,4-D by fish. Rodgers and Stalling[2] studied the uptake and elimination of the ^{14}C-labelled butoxyethanol ester of the herbicide in three species of fish. They reported that the maximum residue concentrations were in the fish within 1 to 2 hr of exposure. Schultz[1] examined the uptake and distribution of ^{14}C-labelled 2,4-D by three species of fish. In these studies, the fish were exposed to the herbicide in plastic pools containing water and a layer of soil at the bottom. The concentration of ^{14}C residues in the edible portion of the fish continued to increase up to 84 days after treatment, but the actual 2,4-D content was negligible indicating that most of the ^{14}C residue was a metabolite(s) of 2,4-D. Because of the way the experiment was designed, it is not possible to assess the role of fish in metabolizing the herbicide. Since 2,4-D is readily degraded by microorganisms,[3] it is not clear whether the fish actually metabolized the herbicide if 2,4-D metabolites were first produced by microorganisms in the water and sediment in the pools and subsequently taken up by fish. In order to

assess the ability of fish to metabolize 2,4-D, the uptake and metabolism of 2,4-D DMA by bluegills and channel catfish were studied under conditions in which microbial degradation of the herbicide in water was minimal.

Material and methods

3. Chemicals. ^{14}C uniformly ring-labelled 2,4-D DMA with a specific activity of 5.38 mCi/mM was purchased from California Bionuclear Corp., Sun Valley, California. This material was judged radiochemically pure by thin-layer chromatography (TLC) in solvent systems consisting of chloroform and chloroform:methanol (1:1 v/v). Nonradioactive 2,4-D DMA was provided by Amchem Products, Inc., Ambler, Pennsylvania.

4. Uptake of ^{14}C 2,4-D DMA. The fish (7.6-10.2 cm (3-4 in.) long) were obtained from the National Fish Hatchery, Orangeburg, South Carolina. They were acclimated to laboratory conditions for 2 weeks before being exposed to 2,4-D. The fish were introduced into fresh spring-water containing 2 ppm of ^{14}C 2,4-D DMA. Each liter (0.26 gal) of water contained two fish and was continuously bubbled with air during the exposure of the fish to the herbicide. Appropriate controls without fish were also included in the study. Two fish were removed from the treated water at 8 hr, 1, 2, 4, 5, and 7 days after treatment, rinsed with clean water three times, and weighed. To determine the amount of radioactivity in the whole body, the fish were cut into small pieces and homogenized with methanol in a Virgis homogenizer. The slurry was shaken for 30 min, then centrifuged; and the supernatant was decanted. The residue was reextracted with 80 percent methanol. After centrifugation, the two extracts were combined and the amount of ^{14}C in the pooled extract was determined by liquid scintillation counting. The amount of ^{14}C in the tissue residue was determined by solubilizing it in a NCS tissue solubilizer (Amersham Searle Corp.) for 48 hr at 50°C (122°F), as described by Sikka et al.[4] Glacial acetic acid (0.003 ml/ml (0.00026/0.00026 gal) of solubilizer) was added to the solubilized tissue, and the solution was counted for ^{14}C using scintillation fluid containing Triton X-100. The samples were stored overnight at 4°C (39.2°F) in the dark before counting. The radioactivities in the methanol extract and

in the tissue residue were combined to calculate the ^{14}C concentration in the fish.

5. To determine the distribution of radioactivity in the fish tissues, the fish were removed from the treated water, rinsed with clean water, and separated into two portions, one containing edible flesh and the other head plus viscera. The amount of radioactivity in the edible flesh was determined using the same procedure described for the whole body, whereas that in the head and viscera portion was measured following solubilization in a NCS tissue solubilizer.

6. Metabolism of 2,4-D. To study the metabolism of 2,4-D by the fish, 30 fish were exposed to 2 ppm of ^{14}C 2,4-D DMA. After 7 days, the fish were removed, rinsed with fresh water, and homogenized with methanol. The homogenate was filtered, and the residue was then extracted with 80 percent methanol. The extracts were combined, and the methanol in the extract was removed under vacuum. The remaining aqueous solution was acidified to a pH of approximately 2 and extracted with chloroform. The chloroform extracts were combined, and the ^{14}C in the organic and aqueous phases was determined. The chloroform extract was concentrated and chromatographed on thin-layer silica gel plates in these solvent systems: (a) chloroform and (b) n-chloroform:methanol (1:1). After drying, the chromatograms were scanned for detection of radioactivity in a Nuclear-Chicago Actigraph. Authenic 2,4-D was cochromated for comparison with unknown metabolites in the extract.

Results

7. Uptake and distribution of ^{14}C 2,4-D DMA by fish.

   a. Bluegills. Table 1 shows the concentration of ^{14}C (expressed as 2,4-D equivalent) in edible flesh, head plus viscera, and total body at various times after exposure to water containing 2 ppm of 2,4-D. The concentrations of ^{14}C-labelled residues reported represent the sum of the radioactivities in the methanol extract and in the extracted residue. The concentration of ^{14}C in the whole fish reached a maximum of about 1 ppm 24 hr after treatment. Longer exposure, up to 7 days, did not result in a significant change in the total ^{14}C concentration. The data showed that the fish removed very small amounts of 2,4-D from the treated water; less than 0.5 percent of the total amount of the herbicide was absorbed by the fish

during a 7-day exposure. At all sampling times, a major portion of the radioactivity absorbed by the fish was associated with the head and viscera portion, which accounted for slightly less than 50 percent of the total fish weight. Also, at all sampling times, less than 5 percent of the total ^{14}C in the fish was associated with the flesh.

b. Channel catfish. The concentration of ^{14}C (expressed as 2,4-D equivalent) in catfish exposed to 2 ppm of ^{14}C 2,4-D is shown in Table 2. As in the case of bluegills, the concentration of radioactivity in the fish reached an equilibrium within 24 hr after treatment. However, catfish removed a smaller amount of the herbicide from the water than bluegills. The maximum concentrations of ^{14}C in catfish and bluegills 24 hr after treatment were 0.20 and 0.93 ppm, respectively.

Table 3 shows the concentration of ^{14}C in edible flesh, head plus viscera, and total body 2 and 7 days after exposure to ^{14}C 2,4-D. As noticed in bluegills, a major portion of the ^{14}C removed by the fish was associated with the head and viscera portion. Edible flesh accounted for about 10 percent of the total ^{14}C residue in the fish.

8. Metabolism of 2,4-D by bluegills and catfish. In the case of bluegills, methanol extracts of edible flesh and head plus viscera were analyzed by TLC to determine the nature of radioactivity, whereas in the case of catfish, the extracts of the whole fish were analyzed. The TLC analysis of the extracts from the bluegills or catfish exposed to ^{14}C 2,4-D for 7 days showed that the ^{14}C in the methanol extractable fraction was present as a single compound that co-chromatographed with authentic ^{14}C 2,4-D in two different solvent systems: (a) Rf 0.04 in chloroform, and (b) 0.71 in chloroform-methanol, 1:1. In contrast to microorganisms that are known to readily degrade 2,4-D,[3] bluegills or catfish do not appear to be capable of metabolizing the herbicide.

9. ^{14}C analysis of the treated water containing fish. The nature of the ^{14}C remaining in the water containing the fish was also determined. After removing the fish, the water was acidified to pH 2 with 1 N HCl and extracted twice with ether. The ether extracts were combined, and the amount of radioactivity in the organic and aqueous phases was determined. The ether extract was concentrated, and aliquots were

chromatographed on thin-layer silica gel plates as described previously. The results showed that essentially all of the radioactivity in the water bathing the fish was extractable with ether. The TLC analysis of the ether extract indicated the presence of only one spot with an Rf value corresponding to that of authentic ^{14}C 2,4-D DMA.

10. Metabolism of 2,4-D by bluegills following intraperitoneal injection. On account of low uptake of 2,4-D by bluegills exposed to the herbicide in water, it was decided to examine the ability of the fish to metabolize 2,4-D administered by intraperitoneal injection. The ^{14}C 2,4-D DMA was dissolved in distilled water, and 50 to 100 µl (0.26×10^{6} gal) of the solution was injected into the peritoneal cavity. The fish were transferred to fresh water, which was periodically monitored for ^{14}C. At the end of the experiment, the water was acidified to pH 2 and extracted with chloroform, and the chloroform and water phases were counted for radioactivity. The chloroform extract was concentrated and analyzed by TLC as described earlier.

11. It was observed that 2,4-D was rapidly excreted from the fish following intraperitoneal injection. About 90 percent of the inital ^{14}C was excreted by the fish within 6 hr of treatment (Table 4). When the water was acidified and extracted with chloroform, essentially all of the ^{14}C was present in the chloroform extract. The TLC of the chloroform extract in two different solvent systems revealed only the presence of 2,4-D.

Discussion

12. The results of this study show that the uptake of 2,4-D DMA by bluegills and channel catfish is very small, and the herbicide does not bioaccumulate in the fish. The residues of 2,4-D detected in the fish in the studies are below the established tolerance limit for 2,4-D of 1.0 ppm in fish.[5] A low uptake of 2,4-D DMA by the fish may be explained by the fact that the herbicide in the water was mostly present in an ionized form, which is less likely to partition from water into fish. Similar results have been reported on the uptake of other water-soluble pesticides and their metabolites.[4,6]

13. The findings demonstrate that the fish were not able to

metabolize 2,4-D. In contrast to these results, Schultz reported that most of the radioactivity in fish exposed to ^{14}C 2,4-D DMA was present as metabolites of the herbicide.[1] However, his studies did not indicate whether the ^{14}C metabolites detected in the fish were produced by the fish themselves, or if the 2,4-D was metabolized outside the fish as a result of microbiological or nonbiological reactions and the metabolites were then absorbed by the fish. Since 2,4-D is known to be readily degraded by microorganisms, it is speculated that the ^{14}C metabolites found in the fish in the studies reported by Schultz originated in the water surrounding the fish as a result of microbial activity and were subsequently removed by the fish. The results reported herein support this speculation. Under the conditions of those experiments in which no degradation of ^{14}C 2,4-D was observed in the water, all the radioactivity in the fish was present as the unchanged herbicide.

Summary

14. Bluegills and channel catfish removed less than 0.5 percent of ^{14}C 2,4-D DMA when exposed in aquaria to water containing 2 ppm of the herbicide. The maximum concentration of 2,4-D in the fish was reached within 24 hr of treatment; thereafter, it did not change significantly up to 7 days. Catfish removed a smaller amount of the herbicide from the water than bluegills. No evidence for bioaccumulation of 2,4-D in the fish was noted during the experiment. A major portion of the radioactivity absorbed by the fish was associated with the head and viscera portions with relatively low concentrations in the edible flesh. The fish did not metabolize 2,4-D during the 7 days following treatment. Bluegills administered ^{14}C 2,4-D DMA by intraperitoneal injection excreted 90 percent of the herbicide within 6 hr of treatment.

Residues of 2,4-D in Blue Crabs

Introduction

15. The U. S. Army Engineer District, Jacksonville, conducts water-hyacinth control operations in the St. Johns River using the 2,4-D DMA. In order to register the herbicide for aquatic plant control and to

assist in establishing residue tolerances for 2,4-D DMA in fish and potable water, the Corps has undertaken projects to obtain information on the residue levels of the herbicide in water, sediment, and fish from the treated areas. This study was undertaken to determine the residues of 2,4-D in blue crabs collected from several locations in the St. Johns River at different times following treatment with the herbicide.

Material and methods

16. The 2,4-D DMA was applied by personnel of the Jacksonville District by means of an airboat at a rate of 2.24-kg acid equivalent per hectare (2 lb/acre) or by aircraft at a rate of 4.48-kg acid equivalent per hectare (4 lb/acre) from July through October. Blue crabs collected periodically from sections of St. Johns River near the water-sampling stations were shipped in dry ice by air to the Syracuse Research Corp. where they were stored frozen until ready for analysis. The edible portion of six crabs collected from a given location was removed, composited, and thoroughly mixed. For each composite sample, triplicate samples each weighing 15-20 g (0.03-0.04 lb) were withdrawn for analysis. The method of analysis[3] used to determine the residues of 2,4-D was essentially the same as described by Schultz.

17. The meat was homogenized with methanol-phosphoric acid (99:1 v/v), and the slurry was suction-filtered through a glass fiber filter. The blender was rinsed with the homogenizing solvent, and the rinse was filtered. The residue was homogenized again with methanol-phosphoric acid, and the homogenate was filtered as before. Fifteen millilitres (0.0039 gal) of water was added to the filtrate, and the solution was evaporated almost to dryness under reduced pressure at 35°C (95°F). The residue was transferred to a separatory funnel with 25 ml (0.0065 gal) water; the solution was acidified to approximately pH 2 and then extracted successively three times with ethyl ether:petroleum ether (1:1). The ether extract was combined, and the aqueous layer was discarded. The ether solution was then extracted with three 25-ml portions of $NaHCO_3$ solution, and the ether layer was discarded. The $NaHCO_3$ solution was acidified to approximately pH 2 with 1 N HCl, extracted with three 25-ml portions of ether, and the ether extracts were combined. After

evaporating the $CHCl_3$, the 2,4-D residue was converted to its methyl ester using diazomethane.[7] The methyl ester of 2,4-D was analyzed by gas chromatography using an electron-capture detector. Operating temperatures for the various components were as follows: inlet, 225°C (437°F); over 185°C (364°F); and detector 280°C (536°F). The chromatographic column was packed with a mixture of equal weights of 4 percent SE-30 and 6 percent QF1 on 80-100 mesh Gas Chrom Q.

18. To determine the percent recovery of 2,4-D from the crab meat, known quantities of the 2,4-D DMA (0.2 and 1.0 ppm) were added to the meat samples. The meat was allowed to equilibrate with 2,4-D for 2-3 hr prior to analysis. The fortified samples then underwent the entire analysis procedure.

Results and discussion

19. Recovery from the meat samples spiked with 0.2 and 1.0 ppm of 2,4-D DMA was about 80 percent as determined by gas chromatographic analysis.

20. Table 5 shows the concentration of 2,4-D in the edible portion of blue crabs collected at different times after application of the herbicide. The average concentration of 2,4-D in the crabs collected in May 1975 ranged from 47.8 to 65.1 ppb. The herbicide could not be detected in the meat of the crabs collected in July, August, and October 1975. The levels of 2,4-D detected in the May samples are within ranges previously reported for blue crabs[8] and are well below the established tolerance limit for 2,4-D of 1.0 ppm in or on fish and shellfish.[5] However, it is not certain that the material that was detected as 2,4-D in the crabs collected in May was in fact 2,4-D and not some other interfering compound, since the herbicide could also be detected in crabs collected at the Guano Wildlife Preserve, an isolated watershed east of the St. Johns River Basin, which has not received any treatment with 2,4-D.

21. The findings of this study demonstrate that application of 2,4-D in flowing waters does not result in an accumulation of the herbicide in blue crabs in levels exceeding the established tolerance limits.

Summary

22. Residues of 2,4-D were determined in blue crabs collected from four locations along the St. Johns River, Florida, following application of 2,4-D DMA. The concentration of 2,4-D in the edible portion of blue crabs collected in May 1975 ranged from 47.8 to 65.1 ppb. However, the herbicide could not be detected in the crabs collected in July, August, and October.

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Table 1

Concentration (ppm) of ^{14}C[2,4-D DMA Equivalent] in Bluegills Exposed to Water Containing 2 ppm ^{14}C 2,4-D DMA*

Time After Exposure	Edible Flesh	Head + Viscera	Total Body
6 hr	0.071	1.084	0.528
1 day	0.078	2.201	0.931
4 days	0.114	1.712	0.868
7 days	0.065	1.501	0.651
14 days	0.096	1.618	0.819

* Two fish analyzed per time interval.

Table 2

Uptake of ^{14}C 2,4-D DMA by Catfish Exposed to 2 ppm of the Herbicide

Time After Exposure	Concentration in Whole Body (ppm expressed as 2,4-D equivalent)
8 hr	0.17
1 day	0.20
2 days	0.20
3 days	0.16
4 days	0.24
5 days	0.25
6 days	0.18
7 days	0.25

Table 3

Distribution of ^{14}C 2,4-D in Catfish Exposed to 2 ppm of the Herbicide

Time After Treatment days	^{14}C Concentration (ppm expressed as 2,4-D equivalent)		
	Flesh	Head + Viscera	Whole
2	0.097		0.25
7	0.064	0.59	0.32

Table 4

Time Course of ^{14}C Excretion Following Intraperitoneal Injection of ^{14}C 2,4-D DMA

Time After Injection hr	^{14}C Excreted (% of ^{14}C Injected)*	
	1.0 ppm Dosage	2.5 ppm Dosage
0-1	62.4	57.7
1-3	22.1	24.0
3-6	4.2	7.2
Total	88.7	88.9

* Average of two fish.

Table 5

Residues of 2,4-D in Blue Crabs of the St. Johns River, Florida

Station	Date	2,4-D Concentration ppb
WC-4	5- 1-75	47.80
	7-22-75	ND*
Welaka	8-25-75	ND
	10- 1-75	ND
	10-20-75	ND
	1-27-76	ND
	5- 1-75	65.10
	7-22-75	ND
WC-3	8-25-75	ND
	10- 1-75	ND
Palatka	10-20-75	ND
	1-27-76	NA**
	5- 1-75	53.40
WC-2	7-22-75	ND
	8-25-75	ND
Green Cove Springs	10- 1-75	ND
	10-20-75	ND
	1-27-76	NA
	5- 1-75	61.00
	7-22-75	ND
WC-1	8-25-75	ND
	9-30-75	ND
Jacksonville	10-20-75	ND
	1-27-76	ND
Guano Wildlife Preserve	5-14-75	62.6

* ND - Not detectable.

** NA - Not applicable (crabs not available at these locations).

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